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Craniofacial Anomalies and Malformations in Respiratory Chain Deficiency

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We report on facial anomalies including round face, high forehead, flat philtrum, apparently low-set ears, and short neck in 4 unrelated patients with mitochondrial respiratory enzyme deficiency. Pre- and postnatal growth retardation with microcephaly, brachydactyly, and hypoplasia of distal and middle phalanges was present in all 4 cases. The diagnosis of respiratory chain deficiency was confirmed by enzymatic and molecular studies. The combination of facial anomalies, prenatal growth failure, and malformations is suggestive of antenatal expression of the disease, and raises the question of the part that respiratory chain deficiencies play in human malformations. © 1996 Wiley-Liss, Inc.

KEY WORDS: respiratory chain deficiency, facial anomalies, prenatal growth failure, malformations

INTRODUCTION

Oxidative phosphorylation, i.e., the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP, occurs in almost all tissues. Consequently, a disorder of oxidative phosphorylation might give rise to any symptom in any organ or tissue at any time, from conception to fetal and postnatal development. In addition, any mode of inheritance is possible, owing to the twofold genetic origin of mitochondrial respiratory enzymes, which are encoded by nuclear genome or by mitochondrial DNA (mtDNA), a maternally inherited 16.5-kilobase pair (kbp) circular genome. Oxidative phosphorylation occurs via five multienzymatic complexes: NADH-coenzyme Q reductase (complex I), succinate CoQ reductase (complex II), CoQ-H_2 cytochrome c reductase (complex III), cytochrome c oxidase (cox, complex IV), and ATP-synthase (complex V).

Recently, we observed minor facial anomalies with pre- and postnatal growth retardation, microcephaly, and multiple malformations in 4 unrelated patients with mitochondrial respiratory chain deficiency. The combination of facial anomalies, prenatal short stature, and malformations in respiratory chain deficiencies raises the question of the part that genetic defects of oxidative phosphorylation play in human malformations.

CLINICAL REPORTS

Patient 1

A girl was born to nonconsanguineous parents at term with birth weight of 2,760 g, length of 46 cm, and head circumference (OFC) of 33 cm. Two older sisters are healthy. Failure to thrive was first noted at age 3 months and rapidly worsened. At age 26 months, weight was 8,300 g (−3 SD), length 75 cm (−4 SD), and OFC 44.5 cm (−2 SD). She also had psychomotor delay: she sat unaided at 15 months, walked at 30 months, and spoke only a few words at 26 months. She was first referred at age 26 months. She had minor facial anomalies including high forehead, flat bridge of nose, flat philtrum, large tongue, apparently low-set ears, and short neck (Fig. 1a). She also had distal limb anomalies with brachydactyly, tapered fingers with small fifth fingernails, abnormal simian creases, and a big gap between the first and second toes. Radiographs showed hypoplasia of distal and middle phalanges and osteopenia (Fig. 1b). There was no anemia, neutropenia, or thrombopenia, no hepatic failure, no exocrine pancreatic dysfunction, no villous atrophy, and no tubulopathy. Results of endocrinological studies were normal, except for a low level of IgF1 (0.06 U/ml; normal, >0.5). Metabolic acidosis (pH 7.29, bicarbonates 14 mM), hyperlactemia (3 mM; normal, <2.4), elevated lactate/pyruvate (L/P) molar ratio (20; normal, <16), and lactaturia were noted.

Patient 2

A boy was born at 34 weeks of gestation to nonconsanguineous healthy parents, with birth weight of 1,670 g and OFC of 28 cm. Oligohydramnios and prenatal

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This study is dedicated to the memory of Jean-Philippe V.

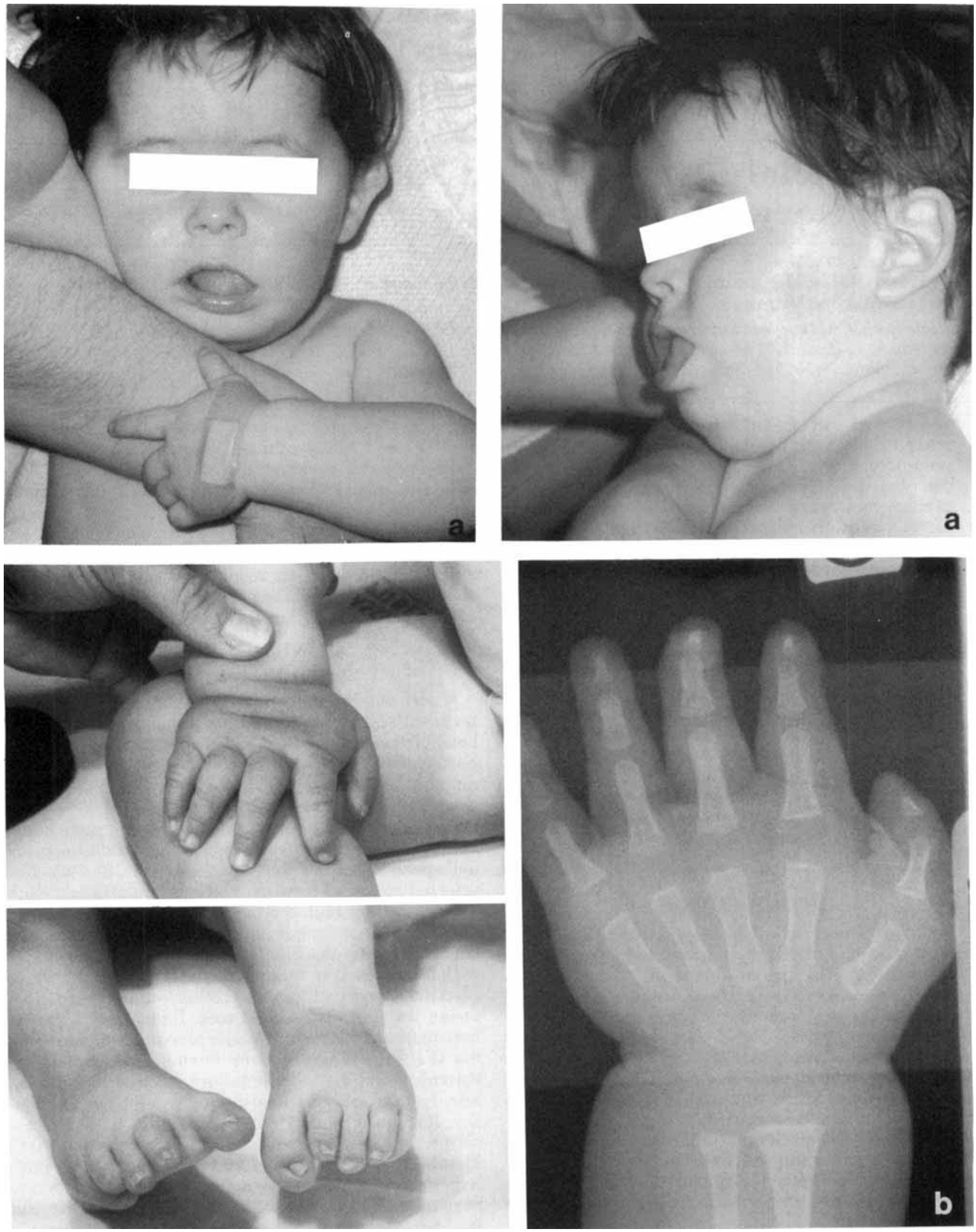

Fig. 1. **a**: Patient 1. Note high forehead, flat bridge of nose, flat philtrum, apparently low-set ears, short neck, and large tongue. **b**: Hand. Note brachydactyly, and distal tapering fingers. Toes. Note big gap between first and the second toes, and abnormal nails. X-ray appearance of left hand. Note hypoplasia of distal and middle phalanges.

short stature were noted during pregnancy. Two older brothers are healthy, but a sister died at age 27 months. She had been born at term with prenatal short stature and hypotrophy (birth weight, 2,380 g). She had psychomotor delay, failed to thrive (−4 SD), and had hepatocellular dysfunction. Several episodes of hypoglycemia with hyperlactemia occurred, and she died at 27 months of hepatic failure associated with lactic acidosis. Patient 2 had early feeding difficulties and frequent vomiting. He was admitted at age 7 months because of failure to thrive (weight, <−4 SD; length, <−4 SD; head circumference, <−4 SD). At that age, he had psychomotor delay, hypotonia, inability to sit unaided, deafness, and bilateral cataract. He also had hepatomegaly with elevated transaminases (ALAT: 149 U/l; normal, <25; ASAT: 165 U/l; normal, <29). He then developed unexplained episodes of dehydration with hypoglycemia and seizures. He was first referred to our unit at age 6 years. On examination, he had a round face, high forehead, apparently low-set ears, flat philtrum, thin vermilion border of upper lip, micrognathia, short neck, and low posterior hairline (Fig. 2). He also had short hands with abnormal simian creases, camptodactyly of the second finger, syndactyly of the second and third toes, and cryptorchidism. He had delayed psychomotor development and no speech. The electroretinogram was altered. Heart, liver, and kidney were normal. Results of endocrinological studies were also normal, except for a low level of IgF1 (0.03 U/ml; normal, >0.5). Metabolic acidosis (pH 7.3, bicarbonates 16 mM) with hyperlactemia (2.6–5.35 mM) and elevated L/P (18–31; normal, <16) and ketone body molar ratios were present (2–4.6, normal, <2). Hyperalaninemia, lactaturia, and abnormal urinary excretion of suberic, adipic, and 3-hydroxy-butyric acids were noted.

Patient 3

A boy was born at 33 weeks of gestation to nonconsanguineous healthy parents (birth weight 1,160 g, head circumference 28 cm). He had 2 brothers, and 1 sister who died in the first days of life with unexplained cardiac insufficiency. He was intubated at birth because of asphyxia. He also had central apnea, hypotonia, poor visual contact, hypertrophic cardiomyopathy, and pancytopenia. At age 2 months, he had minor anomalies including high forehead, downslanting palpebral fissures, micrognathia, short webbed neck, and apparently low-set posteriorly angulated ears suggestive of a CHARGE (Coloboma, Heart defect, Atresia choanal, Retardation, Genital hypoplasia, Ears) association with deficient helix and hypoplastic lobules (Fig. 3). He also had a single palmar crease, and a big gap between the first and second toes. Metabolic investigations revealed permanent hyperlactemia (5–10 mM) with elevated L/P (25–40) and ketone body molar ratios (>3). Hyperalaninemia (500 μM; normal, <274) with urinary excretion of lactic, succinic, fumaric, and 3-hydroxy-butyric acids were noted. He died at age 3 months of an acute episode of metabolic acidosis and collapse.

Patient 4

A girl was born to nonconsanguineous healthy parents at 36 weeks of gestation with a birth weight of 1,390 g. She had 3 healthy brothers and 1 healthy sister. Another sister, born at term (birth weight, 1,700 g), died at age 1 month of diarrhea. At age 13 days, our patient developed severe diarrhea requiring parenteral nutrition. At this time, insulin-dependent diabetes mellitus and cholestasis with liver involvement were noted. She was admitted to our hospital at age 10 months. Weight was 3,400 g (−5 SD), length 55 cm (−6 SD), and OFC 44.5 cm (−3 SD), and she had a high forehead, small nose, flat philtrum, apparently low-set ears, and short neck (Fig. 4). She could not sit and had strabismus. Duodenal atresia with duodenal duplication was also present, and a duodeno-duodenostomy was performed. Histopathological examination of the liver showed microvesicular steatosis and fibrosis, and intestinal biopsy showed partial villous atrophy. Lactate levels (2.6 mM) were mildly elevated, and L/P molar ratios remained in the normal range. Blood ammonemia was consistently elevated (100 μM; normal, <30). Hyperalaninemia (416 μM) with hypocitrullinemia (12 μM; normal, 28) and urinary excretion of fumaric, malic, and 3-hydroxy-butyric acids were noted.

MATERIALS AND METHODS

Biopsy specimens of the deltoid were taken under local anesthesia, and liver specimens were obtained by needle biopsy. Muscle biopsy specimens for light microscope histochemistry were processed according to Dubowitz and Brooke [1973]. Cultured skin fibroblasts and lymphocyte pellets were also obtained. Polarographic and spectrophotometric studies in liver, skeletal muscle homogenate, permeabilized lymphocytes, and skin fibroblasts were carried out as described previously [Chrétien et al., 1990; Rustin et al., 1991]. For Southern blotting, total DNA (5 μg) derived from muscle or lymphocytes was digested, separated by agarose gel electrophoresis (0.7%), and transferred onto nylon filters (Hybond N^+, Amersham International, Amersham, UK). The filters were hybridized with a whole (^{32}P)-dCTP-labeled mitochondrial (mt) DNA probe (4.2 $\times$ 10^6 cpm/ml).

RESULTS

Table I summarizes the respiratory enzyme activities in our 4 patients. A complex IV deficiency was found in the muscle, lymphocytes, and cultured fibroblasts of patient 2, while complex I deficiency was found in the liver of patients 3 and 4. Evidence of complex I deficiency in lymphocytes and skin fibroblasts of patient 3 was based on activity ratios [Rustin et al., 1991]. No respiratory chain deficiency was identified in patient 1, but Southern blot analysis of muscle DNA digested with *Pvu*II (cleavage at position 2652) and hybridization with a whole mtDNA probe showed two populations of mt DNA, one normal (16.5 kb) and one deleted (10 kb). The proportion of deleted mtDNA molecules in patient 1 averaged 15%. In the other 3 patients, Southern blot analysis of mt DNA was normal in absolute amount and size, ruling out depletion and large-scale deletions of the mt genome.

In patient 1, histopathological studies of the muscle showed a predominance of type I fibers and atrophy of

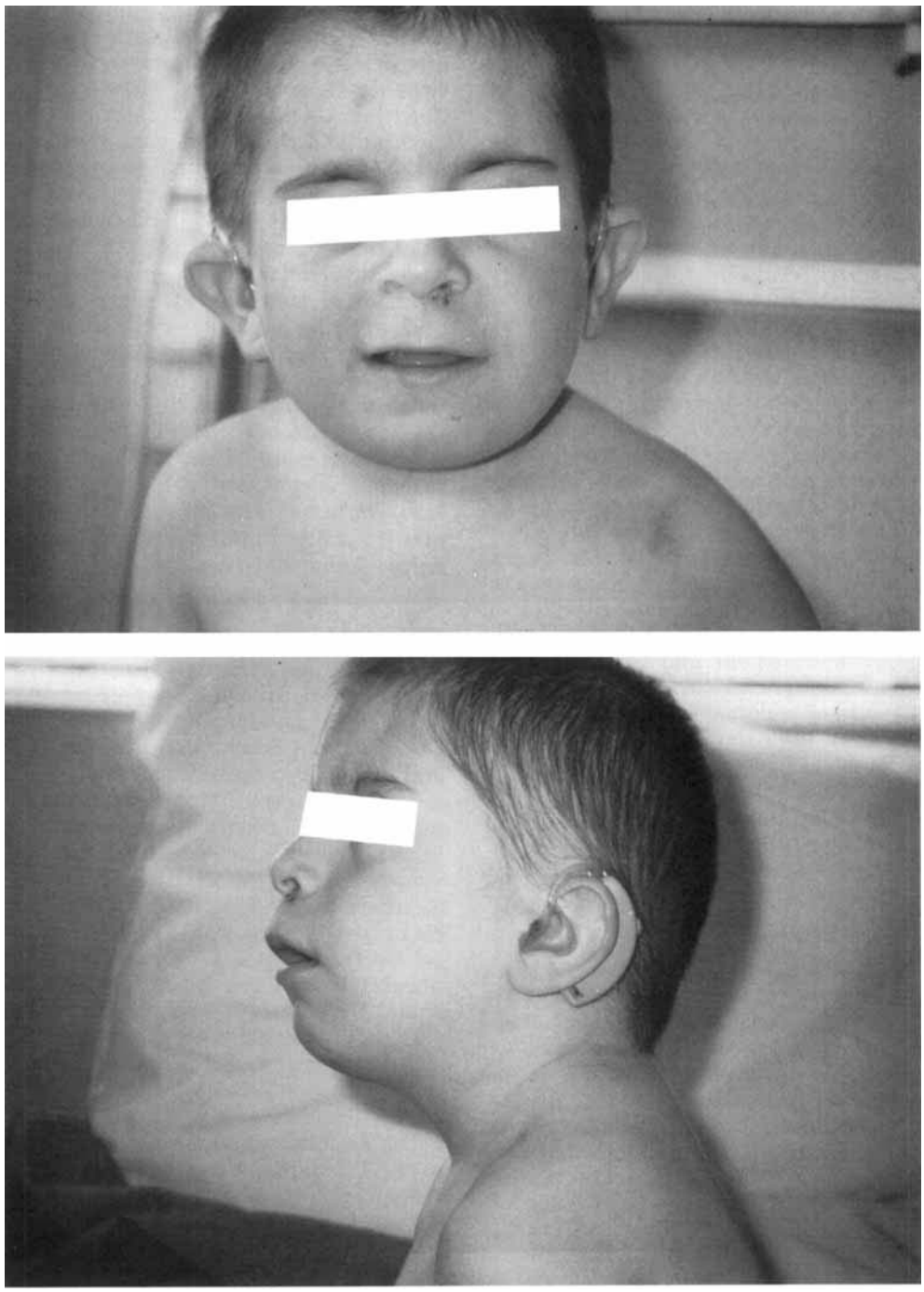

Fig. 2. Patient 2. Note round face, high forehead, flat philtrum, micrognathia, short webbed neck, apparently low-set posteriorly angulated ears with deficient helix, and hypoplastic lobules.

type II fibers (myofibrillar ATPase staining). In patient 2, a predominance of type I fibers with moderate atrophy of type II fibers was also found, and histochemical studies revealed negative cox activity.

DISCUSSION

Here, we report on minor facial anomalies, prenatal growth failure, and malformations in 4 unrelated children with respiratory chain deficiency. The minor anomalies included high forehead, flat philtrum, apparently low-set ears, and short neck. Abnormal simian creases, big gap between first and second toes, short hands, and hypoplastic nails were also present. All patients had severe prenatal intrauterine growth retardation (birth length and weight, <-3 SD) with hypotrophy and microcephaly. Additional malformations are suggestive of

Fig. 3. Patient 3. Note high forehead, downslanting palpebral fissures, micrognathia, short webbed neck, apparently low-set posteriorly angulated ears with deficient helix, and hypoplastic lobules.

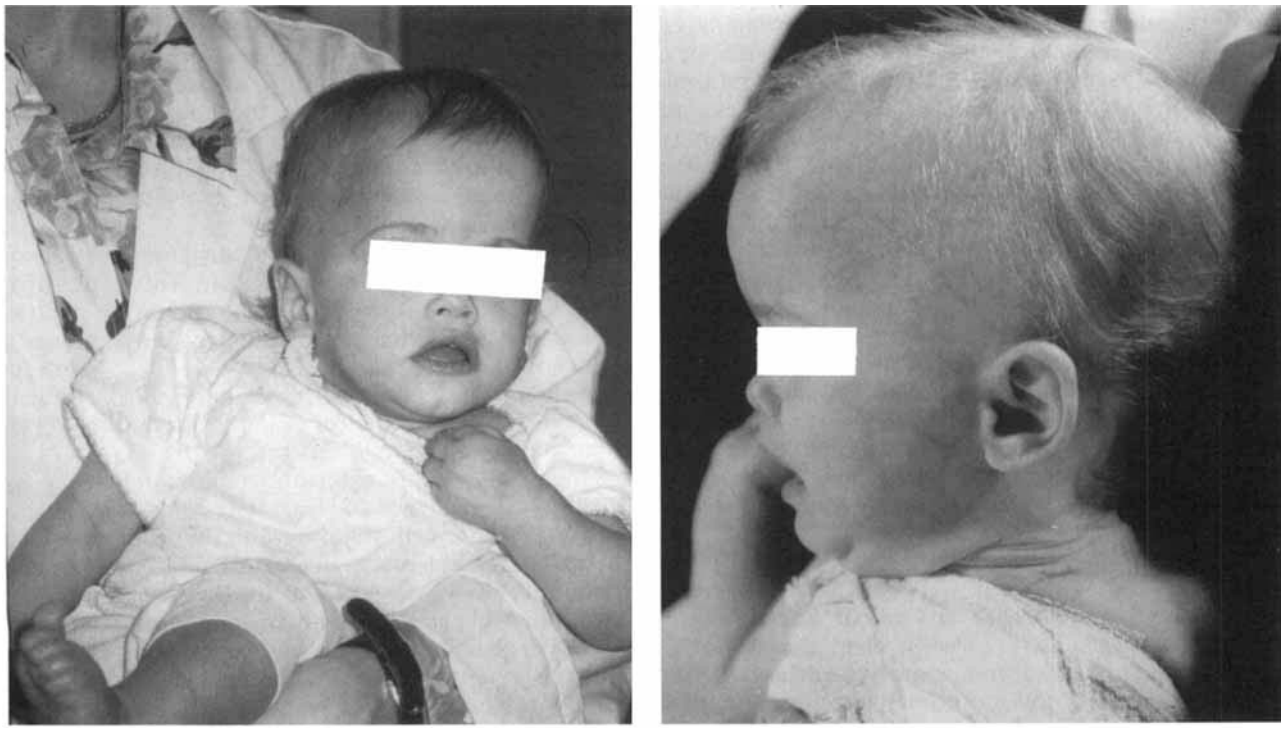

Fig. 4. Patient 4. Note high forehead, small nose, flat philtrum, and short neck.

TABLE I. Spectrophotometric and Oxymetric Analyses of Respiratory Chain in Liver Homogenate, Skeletal Muscle, Circulating Lymphocytes, and Cultured Skin Fibroblasts*

Enzyme activity or oxymetric study	Liver	Muscle	Lymphocytes	Skin fibroblasts
Patient 1	nd	N	nd	N
Patient 2	CIV, 60 nmol/mn/mg protein	CIV, 150 nmol/mn/mg protein	CIV, 30 nmol/mn/mg protein	CIV, 4 nmol/mn/mg protein
Patient 3	CI, 7 nmol/mn/mg protein	nd	Pyruvate oxydase, 4 nmol O_2/mn/mg protein	Pyruvate oxydase, 2.8 nmol O_2/mn/mg protein
			Succinate oxydase, 20 nmol O_2/mn/mg protein	Succinate oxydase, 15.7 nmol O_2/mn/mg protein
			Pyruvate oxydase/succinate oxydase, 5	Pyruvate oxydase/succinate oxydase, 5.7
Patient 4	CI, 9 nmol/mn/mg protein	nd	N	N
Controls (n = 40)	CI, 22–33 nmol/mn/mg protein	CIV, 300–1,340 nmol/mn/mg protein	CIV, 60–216 nmol/mn/mg protein	CIV, 76–153 nmol/mn/mg protein
	CIV, 115–295 nmol/O_2/mn/mg protein		Pyruvate oxydase, 2–9 nmol O_2/mn/mg protein	Pyruvate oxydase, 2–13 nmol O_2/mn/mg protein
			Succinate oxydase, 5–15 nmol O_2/mn/mg protein	Succinate oxydase, 7–21 nmol O_2/mn/mg protein
			Pyruvate oxydase/succinate oxydase, 2 ± 0.2	Pyruvate oxydase/succinate oxydase, 2 ± 0.6

* Enzyme activities are expressed as absolute values in nmol/mn/mg protein. Oxymetric studies have been used for measurement of complex I in lymphocytes and fibroblasts. Results are expressed in nmol O_2/mn/mg protein and as activity ratios. N, normal; nd, not done; C, complex.

antenatal expression of the disease in the skeleton (hypoplasia of distal and middle phalanges), genitalia (cryptorchidism), and the gastrointestinal tract (duodenal atresia).

To date, minor anomalies and malformations have not been reported in respiratory chain deficiency. Some of our findings suggest fetal alcohol syndrome (FAS), namely, minor anomalies, abnormal ears and simian creases, microcephaly, and prenatal short stature [Jones and Smith, 1973]. These anomalies common to FAS and metabolic diseases have been described in pyruvate dehydrogenase (PDH) deficiency [Robinson et al., 1987]. It has been proposed that in FAS, acetaldehyde, a product of alcohol oxidation, might inhibit the PDH complex and be responsible for a secondary PDH deficiency in utero. The deterioration of mitochondrial function evidenced by morphological and functional studies is one possible mechanism of alcohol-related diseases. Indeed, chronic ethanol intake leads to a marked dysfunction of electron transport components and of ATP synthase [Hoek, 1994]. Alternative hypotheses include the antenatal expression of respiratory chain deficiency or intoxication of the fetus by abnormal intermediates in utero.

Developmental anomalies and minor anomalies in metabolic diseases have already been described in Smith-Lemli-Opitz syndrome, mevalonic aciduria, carbohydrate deficiency glycoprotein syndrome, and peroxisomal disorders [Smith et al., 1964; Hoffmann et al., 1993; Jaeken et al., 1991; Passarge et al., 1967]. Two general mechanisms might be involved in malformative metabolic syndromes, namely, dysplasia and disruption [Spranger et al., 1982]. Dysplasia corresponds to the morphological result of an abnormal organization of tissue cells, and may be caused by a peroxisomal disorder. On the other hand, disruption corresponds to a morphologic defect of an organ, resulting from the breakdown of an originally normal developmental process. It may be caused by alcohol or other maternal intoxications. Both mechanisms could account for the developmental anomalies reported in our respiratory enzyme deficiency patients.

Why minor anomalies are not more frequent in respiratory chain deficiencies is not clear. This could be related to the time course of mutated enzyme gene expression or to the interplay of maternal, environmental, and/or nutritional factors. However, since diagnosing respiratory chain deficiencies is particularly difficult, the contribution of this condition to human malformations might well be underestimated.

The diagnosis of respiratory chain deficiency can hardly be considered early, especially when the presenting symptom is the only one found. By contrast, this diagnosis is a more obvious possibility when seemingly unrelated symptoms occur. Based on this observation and bearing in mind the ubiquitous nature of oxidative phosphorylation, we suggest giving consideration to genetic defects of the mitochondrial energy supply in elucidating the origin of other diseases with seemingly unrelated symptoms, including minor facial and developmental anomalies.

NOTE ADDED IN PROOF

Since the submission of this manuscript, an article by Damian MS et al. reported VACTERL with the mitochondrial NI 3243 point mutation.

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